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PCR detection of thymidine kinase gen of latent herpes simplex Virus type 1 in mice trigeminal ganglia

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ABSTRACT

Herpes simplex virus type 1 establishes a latent infection in the peripheral nervous system following primary infection. During latent infection, virus genome exhibit limited transcription, with the HSV LATs consistently detected in latency infected ganaglia. Following ocular infection viral latency develops in the trigeminal ganglia. In this study PCR has been used for detection of HSV-1 nucliec acid . BALB/c mice were inoculated with HSV-1 and infected mice ganglia were applied to detect HSV-1 TK gene. Amplification of TK fragment in mice ganglia was considered as an indicator of viral latency. The results were suggested that TK PCR can be used as a reliable diagnostic tool and it is a specific and highly sensitive method for the detection of HSV genome in trigeminal ganglia.

Keywords: Herpes simplex virus, Latency, Trigeminal ganglia, Thymidine kinase

INTRODUCTION

Herpes simplex viruses type 1 and 2 (HSV-1 and HSV-2) are common throughout the world. HSV invades the host nervous system through the innervating sensory nerve terminals at the site of primary infection (Feldman *et al* 2002, Shimeld *et al* 1999). In the sensory ganglia, acute viral replication is resolved within about 10 days post inoculation. Despite complete clearance of infectious virus from the ganglia, in the small population of neurons the viral genome remains in a transcriptionally repressed state within the nucleus as an episome (Whitley *et al* 2001). Latent infection is a hallmark of herpes simplex virus (HSV) infection in human and in a number of animal models. It is characterized by the presence of viral genome in neurons of sensory nerve ganglia enervating the sites of initial viral infection in the epithelium (Wagner *et al* 1997). Following infection, the virus travels up nerves and establishes latent infection in neurons of the trigeminal ganglia (Perng *et al* 1996). In neurons during the latent phase, the majority of viral genomes are transcriptionally quiescent (Wagner *et al* 1997). Under certain conditions which include stress or exposure to UV light, the virus may reactivate, travel back down the nerve and cause recurrent infection. (Leib *et al* 2002). The herpes simplex virus type 1 (HSV-1) encoded

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thymidine kinase (TK) plays a critical role in acute viral replication or reactivation of virus from latent ganglia (Wang *et al* 2003, Jacobson *et al* 1993, Masayuki *et al* 2002). This could be related to its biological property of being able to process multiple kinase activities. It can be good candidate for monitoring in latent HSV-1 infection of mice ganglia (Coen *et al* 1989, Balzarini *et al* 1995). The standard virological methods used for detection of latent HSV lack considerable sensitivity and are often time consuming (Madhavan *et al* 1999). Therefore, we standardized PCR using the primers, which amplified a 398bp product of HSV-1TK gene. Due to the high sensitivity, specificity and accuracy of the PCR, this technique was used for detection of HSV latent infection. (Daniel *et al* 2002, Knox *et al* 1998).

MATERIALS AND METHODS

Mice Infections. Six to Seven weeks old female BALB/c mice (7 mice per group) were obtained from RAZI institute, (Karaj, Iran) with an average weight of 20 g were anesthetized by an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg), and both corneas were slightly scarified using a 26 gauge needle. A group of mice were then inoculated with tissue culture medium alone (mock), or with wild-type virus at a dose of 2 x 10^6 $TCID_{50}$/eye. The lid was held closed and rubbed gently for 30 sec.

HSV-1 DNA extraction from cell cultures. A wild HSV-1 isolate was grown in Vero cell lines until 80-90% of cell showed cytopathic effect (CPE). The inoculated cells were harvested and subjected to three rounds of freezing and thawing. Equal volumes of the harvested virus and lysis buffer [0.l M Tris-Cl (pH 8.0), 0.01 M EDTA, 1% Sodium dodecyl sulphate and 25.8 m1 of proteinase K (20 mg: ml)] were incubated at 42 °C for 4 hrs. The mixture was vortexed every 30 min, then inactivated at 94 °C for 10 min, and followed by standard phenol chloroform extraction method. The extracted DNA was precipitated with ethanol and finally dissolved in 20 µl of distilled water. The extracted DNA was used as control for optimization of PCR. Vero cells were subjected to DNA extraction that used as a negative control.

Trigeminal ganglia DNA extraction. At 30 days post inoculation, mice were sacrificed and trigeminal ganglia were removed and rapidly frozen in liquid nitrogen. DNA was extracted from total target tissue. Briefly, frozen tissue chopped in to small pieces and then suspended in 3 ml of DNA extraction buffer (10 mM Tris [pH 7.5], 25 mM EDTA, 10 mM NaCl), then 100 ml of 20% sodium dodecyl sulfate and 100 ml of proteinase K (20 mg/ml) were added, and the treated tissue was digested at 56 °C overnight. The DNA solution was extracted three times with phenol chloroform and once more with chloroform and then precipitated with ethanol overnight. It was deposited by centrifugation, and finally, dissolved in 20 µl of deionised distilled water.

Polymerase chain reaction. The standard PCR, using Taq DNA polymerase (15), was modified to permit qualification of HSV DNA. The details of the procedure, including the measures to preclude contamination with exogenous DNA sequences standards, were considered. Known amounts of HSV DNA were mixed with 40 pmol primer sets. The primers, CT TAACAGCGTCAACAGCGT and CAAAGAGGTGCGGGAGT, were designed by Generunner and used to amplify a 398 base fragment of TK. These DNAs were then assembled into 100 µl reactions containing 50 mM KCl, 10 mM Tris chloride (pH 8.4), 4.5 mM $MgCl_2$ (which was found to be optimal for this primer-template combination), 200 µM concentrations of each deoxynucleoside triphosphate, and 4 U of Taq DNA polymerase. PCR amplification was then performed for 32 cycles, with denaturation for 5 min at 94 °C, annealing for 1 min at 62 °C, and extension for 1 min at 72 °C, with a final additional extension of 7 min.

Sensitivity and Specificity of PCR. The sensitivity of PCR was determined with standard strains of HSV-1(KOS). The DNA extracted from infected tissue culture was serially diluted in 10-fold dilutions and then subjected to PCR. The concentration of extracted DNA was determined spectrophotometrically at 260 nm. The sensitivity of PCR with standard strain of virus was calculated using different set of primers, which amplify 398 bp fragment or full length HSV-1TK. Specificity of HSV-1 DNA were amplified using target primers versus non HSV-1 DNA such as normal cell or ganglia with the same optimized conditions.

Analysis of PCR products. The amplified products were subjected to gel electrophoresis using 2% agarose gel that it was stained with ethidium bromide (Sambrook *et al* 1989). The gel was read at 302 nm on an UV illuminator. The amplified DNA was visualized by an UV trans-illuminator at 302 nm.

RESULTS

Sham-infected mice showed no signs of HSV-1 infection**.** Standard positive and negative DNA samples for PCR were prepared from HSV-1 infected and uninfected Vero cells, respectively. Blanks were always included during PCR to check that no cross- contamination had occurred. The correct size of DNA fragment (398) was observed in positive control containing HSV-1 while no DNA amplified in Vero cell line as negative control. A 398 bp fragment was amplified when inoculated Vero cell culture were tested in PCR using primers selected from the HSV-1 TK gene sequence (Figure 1). Amplification of an expected DNA fragment (398 bp) from infected Vero cells as well as infected mice ganglia indicated that PCR reaction can be performed for detection of latent HSV-1 TK gene. The sensitivity of initial PCR was determined by performing a PCR on serial dilutions of extracted DNA for first step of optimization. Results showed 25 ng of total DNA is detectable by PCR, for compari-

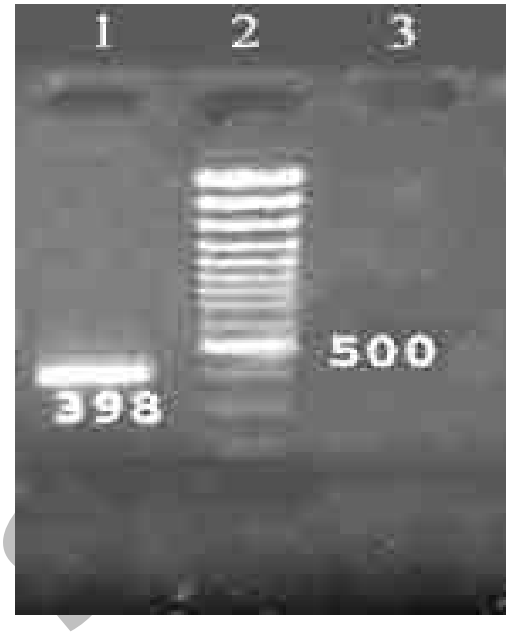

Figure 1. Optimization of PCR conditions using positive and negative controls. A396 bp fragment was observed in a 1.5% agarose gel. *Lane 1:* DNA size marker. *Lane 2:* HSV-1 infected vero cells as a positive control and *Lane 3:* uninoculated Vero cell culture as negative control.

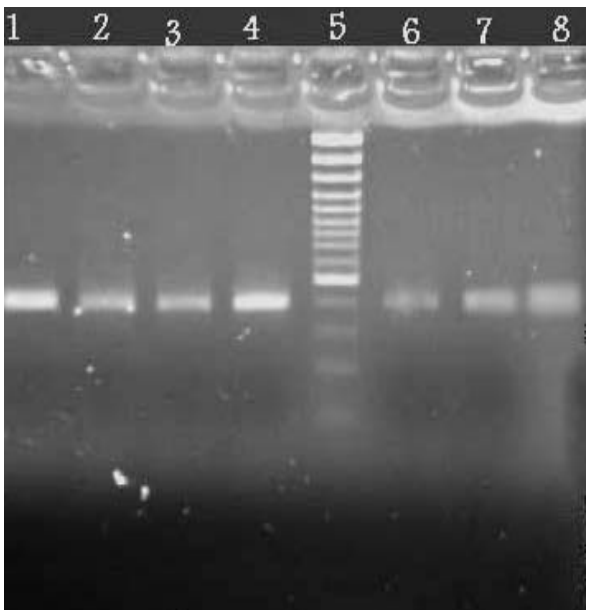

Figure 2. Thymidine kinase 398 bp fragment in trigeminal ganglia. *Lanes 1, 2, 3,4,6,7 and 8:* viral DNA from trigeminal ganglia and *Lane* 5: DNA size marker.

son of initial (non-optimized) and optimized PCR, two sets of experiments with similar amount of template DNA were prepared and PCR was performed under two different conditions, one with optimized PCR and one with initial PCR. Using different set of primers sensitivity of these PCRs were determined by amplification's ability of target gene on serial dilutions of DNA. Comparison of initial and optimized HSV-1 PCR showed that the sensitivity of optimized PCR was much higher than the initial PCR. According to tested subjects, sensitive PCR enabled to detect the TK gene in 7 infected mice (Figure 2). For specificity evaluation we did not get false positive with any other non HSV-1 DNA which was used in this study.

DISCUSSION

The discovery that administration of HSV-1 at various sites caused acute infection and later, establishment of a latent infection in the PNS of mice, leads to usage of mice as a model for latency of the virus in the PNS. Studies on the mouse central nervous system (CNS), using explanation and co-cultivation, or molecular hybridization, showed that after HSV-1 infection, viral latency could be established in the brain. Although, theoretically, a single infectious viral particle is sufficient for virus isolation, in practice the infectious virus titer is markedly reduced during storage of specimens resulting in false negativity by standard virological investigations (Madhavan *et al* 1999). Evaluation of latency can be done by detection of LAT and hybridization based RNA, but the currently available data show that neurons contain more viral DNA than LAT transcripts. It seems that for detection of latency in HSV infection, PCR provide more accurate data than RT PCR and it is more rapid, easy and available (Perng *et al* 1996). The current study demonstrates that the presence of TK could be an important latency marker for diagnosis of HSV latent state. In conclusion TK-specific primers which amplify shorter products much more efficient than those of amplify full length TK-gene. In addition to these facts that shorter sequences can be amplified more efficiently, in the case of HSV genome with high GC content, and limited taq polymerase proccesivity, we amplified the shorter product. Analysis of HSV genome in TG could facilitate the prediction of reliable prognosis which in turn leads to treatment of HSV infection infection in special issues such as immunocompromised state or in explanation.

References

Balzarini, J.K.W., Morin, E.E., Knaus, L., Wiebe, I. and Clercq, E.D. (1995). Novel (E)-5-(2-iodovinyl)-29-deoxyuridine derivatives as potential cytostatic agents against herpes simplex virus thymidine kinase gene transfected tumors. *Gene Therapy* 2: 317-322.

Bustos, D.E. and Atherton, S.S. (2002). Detection of Herpes Simplex Virus Type 1 in Human Ciliary Ganglia, *Investigative Ophthalmology & Visual Science* 43(7): 458-466.

Coen, D.M., Kosz-Vnenchak, M., Jacobson, J.G., Leib, D.A., Bogard, C.L., Schaffer, P.A., Tyler, K.L. and Knipe, D.M. (1989). Thymidine kinase-negative herpes simplex virus mutants establish latency mouse trigeminal ganglia but do not reactivate. *Proceedings of the National Academy of Sciences* 86: 4736-4740.

Feldman, L.T., Ellison, C., Voytek, C., Yang, L., Krause, P. and Margolis, T.P. (2002.). Spontaneous molecular reactivation of herpes simplex virus type 1 latency in mice. *Proceedings of the National Academy of Sciences* 99: 978-983.

Jacobson, J.G., Ruffner, K.L., Kosz-Vnenchak, M., Hwang, C.B.C., Wobbe, K.K., Knipe, D.M. and Coen, D.M. (1993). Herpes simplex virus thymidine kinase and specific stages of latency in murine trigeminal ganglia. *Journal of Virology* 67: 6903-6908.

Knox, C.M., Chandler, D., Short, G.A. and Margolis, T.P. (1998). Polymerase chain reaction-based assays of vitreous samples for the diagnosis of viral retinitis. *Ophthalmology* 105: 37-45.

Leib, D.A. (2002). Counteraction of interferon-induced antiviral responses by herpes simplex viruses. *Current*

Topics in Microbiology and Immunology 269: 171-185.

Madhavan , H.N. , K. Priya, A.R. Anand, K. and Therese, L. (1999). Detection of Herpes simplex virus (HSV) genome using polymerase chain reaction (PCR) in clinical samples Comparison of PCR with standard laboratory methods for the detection of HSV. *Journal of Clinical Virology* 14: 145-151.

Masayuki, S.A., Suzutani, B.T., Clercq , E.D. and Masahiro, N. (2002). Genotypic and phenotypic characterization of the thymidine kinase of ACV-resistant HSV-1 derived from an acyclovirsensitive herpes simplex virus type 1 strain. *Antiviral Research* 56: 253-262.

Perng, G., Ghiasi, H., Slavina, S.M., Nesburn, A.B. and Wechsler, S. (1996). The spontaneous reactivation function of the herpes simplex virus type 1 LAT gene resides completely within the first 1.5 kilobases of the 8.3 kilobase primary transcript. *Journal of Virology* 70: 976-984.

Shimeld, C., Easty, D.L., and. Hill, T.J. (1999). Reactivation of herpes simplex virus type 1 in the mouse trigeminal ganglia: an in vivo study of virus antigen and cytokines. *Journal of Virology* 73: 1767-1773.

Wagner, E.K., and Bloom, D.C. (1997). Experimental investigation of herpes simplex virus latency. *Clinical Microbiology Reviews* 10: 419-443.

Wang, Y.T., Wang, Y.A., Lu, Q., Charles, B.C. and Hwang, C.B. (2003). Thymidine kKinase of herpes simplex virus type 1 strain KOS lacks mutator activity. *Virology* 305: 388-396.

Whitley, R.J. (2001). Herpes simplex viruses. In D.M. Knipe, P.M. Howley, D.E. Griffin, R. A. Lamb, M. A. Martin, Roizman, B. and Straus, S.E. (4th edn.), Volume 2, *Fields virology.* Pp: 2461-2510. Lippincott Williams & Wilkins, Philadelphia, Pa.